Cell Reports

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Subunits of the Histone Chaperone CAF1 Also Mediate Assembly of Protamine-Based Chromatin

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http://dx.doi.org/10.1016/j.celrep.2013.06.002

SUMMARY

One of the most dramatic forms of chromatin reorganization occurs during spermatogenesis, when the paternal genome is repackaged from a nucleosomal to a protamine-based structure. We assessed the role of the canonical histone chaperone CAF1 in *Drosophila* spermatogenesis. In this process, CAF1 does not behave as a complex, but its subunits display distinct chromatin dynamics. During histone-to-protamine replacement, CAF1-p180 dissociates from the DNA while CAF1-p75 binds and stays on as a component of sperm chromatin. Association of CAF1-p75 with the paternal genome depends on CAF1-p180 and protamines. Conversely, CAF1-p75 binds protamines and is required for their incorporation into sperm chromatin. Histone removal, however, occurs independently of CAF1 or protamines. Thus, CAF1-p180 and CAF1-p75 function in a temporal hierarchy during sperm chromatin assembly, with CAF1-p75 acting as a protamine-loading factor. These results show that CAF1 subunits mediate the assembly of two fundamentally different forms of chromatin.

INTRODUCTION

Chromatin dynamics are fundamental to virtually all aspects of eukaryotic genome biology. The nucleosome, comprising 147 bp of DNA wrapped tightly in ~1.7 left-handed superhelical turns around a core histone octamer of H2A, H2B, H3, and H4, is the basic unit of eukaryotic chromatin (Kornberg, 1977; Luger et al., 1997). Histone chaperones are crucial mediators of nucleosome assembly and disassembly (De Koning et al., 2007; Eitoku et al., 2008; Hondele and Ladurner, 2011; Park and Luger, 2008; Ransom et al., 2010). They guide the trafficking of newly synthesized histones and deposit them onto DNA during replication-coupled chromatin assembly. In addition, histone chaperones play a variety of regulatory roles in chromosome biology.

Chromatin assembly factor 1 (CAF1) is a canonical histone H3/H4 chaperone that mediates replication-coupled nucleosome assembly (Gaillard et al., 1996; Kaufman et al., 1995; Smith and Stillman, 1991; Verreault et al., 1996). CAF1 comprises three evolutionarily conserved subunits, named after their apparent molecular weights. Human CAF1 is composed of p150, p60, and p48, which correspond to *Drosophila* p180, p105/p75, and p55, respectively. CAF1-p75 is encoded by the same gene as CAF1-p105 and is the result of proteolytic processing (Figure 1A; Tyler et al., 2001). Human CAF1-p48 and fly CAF1-p55 are present in a multitude of chromatin-modulating complexes, of which CAF1 probably forms only a minor portion. Human CAF1-p150 and CAF1-p60 suffice for CAF1 activity and binding of newly synthesized histones (Kaufman et al., 1995).

Germ cell differentiation yields highly specialized haploid cells that are totipotent and can give rise to a new organism. Male gametogenesis involves an amazing degree of cellular transformation and genomic reorganization (Fuller, 1998; Kimmins and Sassone-Corsi, 2005; Sassone-Corsi, 2002; White-Cooper, 2010). In mammals and insects, the nucleosomal organization of the paternal genome is replaced by a highly condensed, protamine-based structure (Braun, 2001; Jayaramaiah Raja and Renkawitz-Pohl, 2005; Kimmins and Sassone-Corsi, 2005; Sassone-Corsi, 2002). Protamine-based chromatin forms a toroidal, densely compacted architecture that is fundamentally different from the nucleosomal arrays of somatic cells. The repackaging of the haploid genome enables a striking compression of the nucleus, which promotes sperm motility and helps maintain the integrity of the paternal genome. After completion of *Drosophila* spermiogenesis, the nuclear volume is about 200-fold smaller than that of a typical somatic cell (Fuller, 1998; Tokuyasu, 1974; White-Cooper, 2010).

Protamines are testes-specific, highly basic proteins that are believed to be related to linker histone H1 (Eirín-López et al., 2006). In addition to mediating chromatin compaction, protamines protect sperm DNA from physical damage or mutagenesis. Protamines are a diverse and rapidly evolving class of proteins. The *Drosophila* male-specific transcript 35Ba (*Mst35Ba*) and *Mst35Bb* genes encode Protamine A and B (ProtA and ProtB), which show homology to mammalian protamines Prot1 and Prot2 (Andrews et al., 2000; Jayaramaiah

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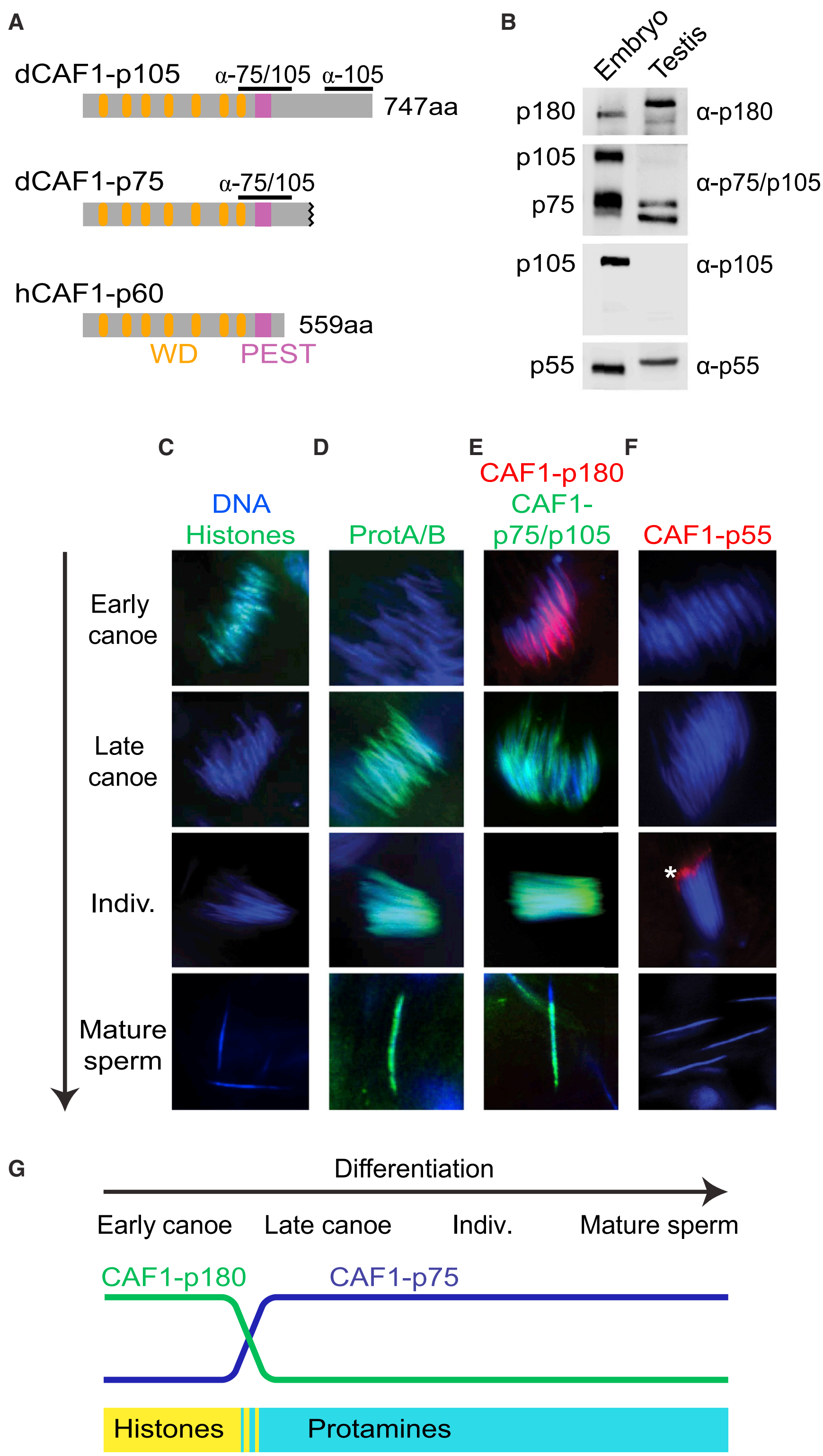

Figure 1. Distinct Chromatin Dynamics of CAF1 Subunits during Spermatogenesis

(A) Predicted structure of *Drosophila* dCAF1-p75 and dCAF1-p105 and human hCAF1-p60. WD repeats and PEST domains are indicated. The underlined regions mark the polypeptides used to generate either α-p75/105- or α-p105 antibodies.

(B) Immunoblotting analysis of CAF-1 in embryo or testis extracts. We used antibodies that recognize both CAF1-p75 and CAF1-p105 (α-p75/105) or only the p105 isoform (α-p105). CAF1-p105 is readily recognized in embryo extract but undetectable in testis.

(C–F) Chromatin binding of CAF1 subunits during the four major postmeiotic stages of spermatogenesis: early canoe, late canoe, individualization (Indiv.), and mature sperm. In *Drosophila*, before individualization, 64 synchronously developing sperm heads are arranged in parallel in one cyst. (C and D) Shown is the histone-to-protamine transition from early to late canoe visualized by immunofluorescence using antibodies to detect endogenous histones or protamine A and B (ProtA/B). DNA is visualized by DAPI staining (blue). (E) CAF1-p180 (red) is chromatin associated at early canoe, whereas CAF1-p75 (green) binds at late canoe and persists associated with mature sperm DNA. (F) CAF1-p55 does not associate with chromatin during spermatogenesis. The staining indicated with an "*" does not correspond to a known structure. For all panels, images of separate channels are shown in Figure S1.

(G) Summary of CAF1 subunit dynamics during the histone-to-protamine transition.

See also Figure S1.

Raja and Renkawitz-Pohl, 2005; Russell and Kaiser, 1993). Mst77F is another spermatid-specific histone H1-like protein, with homology to mammalian HILS, which also replaces the histones during chromatin condensation (Jayaramaiah Raja and Renkawitz-Pohl, 2005). Mst77F is essential for *Drosophila* male fertility, whereas ProtA and ProtB are surprisingly dispensable, although they do help protect the paternal genome from DNA damage (Rathke et al., 2010).

Here, we assessed if some of the factors dedicated to chromatin (dis)assembly in somatic cells might also function in chromatin repackaging during spermatogenesis. We found that the classic replication-dependent histone chaperone CAF1 plays a crucial role during the histone-to-protamine transition. Our results suggest that CAF1-p75 acts as an essential protamine-loading factor. Thus, histones are not the only substrate of histone chaperones. Although protamine-based chromatin is fundamentally different from nucleosomal chromatin, their assembly involves components of the same cellular machinery.

RESULTS

Different CAF1 Subunits Display Distinct Chromatin Dynamics during Spermatogenesis

To visualize CAF1 during spermatogenesis in *Drosophila*, we generated antibodies directed against individual CAF1 subunits. To distinguish between CAF1-p105 and CAF1-p75, we used antibodies that are selective for p105 and antibodies that recognized both p105 and p75 (Figure 1A). We analyzed CAF1 in extracts prepared from either 0–12 hr old embryos or testes dissected from wild-type males by western immunoblotting (Figure 1B). The most striking difference between embryonic CAF1 and testicular CAF1 is the apparent absence of CAF1-p105 in testes. In addition, the migration of p180, p75, and p55 isolated from testes is somewhat different from their counterparts in embryo extract, suggesting they might be encoded by alternative transcripts or subjected to posttranslational processing.

We monitored CAF1 chromatin-binding dynamics during the repackaging of the male genome from a nucleosomal- to a protamine-based structure (Figures 1C–1G; for single channel images, see Figure S1). We concentrated on postmeiotic stages, starting with early and late canoe, followed by individualization and, finally, the formation of mature sperm. Early canoe is defined by the start of histone removal, whereas late canoe is marked by protamine accumulation. Indeed, immunofluorescence using antibodies raised against core histones detected endogenous histones at early but not at late canoe stages (Figure 1C). To visualize endogenous *Drosophila* protamines, we generated antibodies against ProtA and ProtB. Because both antisera yielded similar results, we combined them to detect ProtA and ProtB simultaneously. Decoration of the male genome with ProtA/B was readily observed from late canoe onward, including mature sperm (Figure 1D). The anti-ProtA/B antibodies did not stain the sperm DNA from ProtA/B null males, demonstrating their specificity (Figure S1C). Thus, between early and late canoe stages, protamines replace the bulk of histones. We note that in costaining experiments, we never detected a mixture of histones and protamines on the DNA, suggesting that their exchange is a relatively fast, all-or-none event.

Next, we compared the chromatin association of individual CAF1 subunits. At early canoe stages, CAF1-p180 (red) is DNA associated whereas CAF1-p75 is not (Figure 1E). By late canoe, however, CAF-p180 has dissociated whereas CAF1-p75 (green) now decorates the paternal genome and remains bound in mature sperm. Thus, during the histone-to-protamine transition, there is also an exchange between CAF1-p180 and CAF1-p75. Confirming the absence of CAF1-p105 in testes (Figure 1B), the p105-specific antibody did not recognize chromatin at any stage (data not shown). In contrast to embryos, we could not observe chromatin-bound CAF1-p55 during spermatogenesis (Figures 1F and S1G). Collectively, these results show that during spermatogenesis, CAF1 does not function as an integral complex. Instead, each CAF1 subunit has its individual dynamics of association with the paternal genome (Figure 1G). CAF1-p180 binds chromatin from the round nuclei (Figure S1F) through early canoe stages. Whereas we did not detect CAF1-p105 in testis, CAF1-p75 associates with chromatin at the histone-to-protamine transition and is a component of mature sperm chromatin. Finally, CAF1-p55 does not appear to be directly involved in the histone-to-protamine transition. We conclude that CAF1-p180 and, in particular, CAF1-p75 might participate in the repackaging of the paternal genome during spermatogenesis.

CAF1-p75, but Not Other CAF1 Subunits, Binds Protamines

The concurrent assembly of protamines and CAF1-p75 into sperm chromatin made us wonder whether they might interact physically. To test this idea, we used antibodies against CAF1-p75/p105 to immunopurify p75 and p105 from testis and embryo extracts. Note that the testis extract was derived from a mixture of somatic and germline cells. Following extensive washes with a buffer containing 600 mM KCl and 0.1% NP-40, the identities of the isolated proteins were determined by mass spectrometry (Table S1). In addition to the other CAF1 subunits and histone H4, ProtA and ProtB were found to be associated with CAF1-p75 isolated from testis extract. Next, we immunopurified protamines from testis extract and tested the association of CAF1 subunits. Following extensive washes, bound material was resolved by SDS-PAGE and analyzed by protein immunoblotting (Figure 2A). Of the three CAF1 subunits, only p75 copurified with the protamines. In contrast, immunopurification of histones from embryo extract yielded the full complement of CAF1 subunits (Figure 2B). Incubation of testis extract with recombinant GST-ProtA also revealed the selective binding of CAF1-p75 but not the other CAF1 subunits (Figure 2C). Interestingly, we obtained a similar result using embryo extract, suggesting CAF1-p75, but not CAF1-p105 or the full CAF1 complex, can bind to protamines (Figure 2D). Sephacryl S-300 size-exclusion chromatography of embryo extract revealed that substantial fractions of CAF1-p75 and CAF1-p105 exist separate from the canonical CAF1 complex (Figure 2E). In conclusion, CAF1-p75 also acts outside the CAF1 complex and binds protamines independently (Figure 2F).

CAF1-p75 Association with Sperm Chromatin Is Dependent on CAF1-p180

During spermatogenesis, CAF1-p180 binds chromatin prior to CAF1-p75. When CAF1-p75 appears on the paternal chromosomes during late canoe, CAF1-p180 dissociates (Figure 1). To test whether chromatin binding of CAF1-p75 was dependent on CAF1-p180, we employed the GAL4 upstream activating sequence (UAS) system in *Drosophila* (Brand and Perrimon, 1993). We used the C135 GAL4 enhancer trap line that drives expression of the GAL4 transcriptional activator in the male reproductive tract (Hrdlicka et al., 2002). By combining the driver line with UAS-controlled transgenes expressing double-stranded RNAs directed against *Caf1-p75* (C135 > p75^{RNAi}), *Caf1-p180* (C135 > p180^{RNAi}), or *Caf1-p55* (C135 > p55^{RNAi}) messenger RNA, we could selectively deplete p75, p180, or p55 during spermatogenesis (Figures 3 and S2). Knockdown of CAF1-p180 led to a loss of CAF1-p75 association with sperm chromatin (Figure 3A). In contrast, depletion of CAF1-p75 did not affect chromatin incorporation of CAF1-p180 (Figure 3B). Knockdown of CAF1-p55 had no effect on the chromatin dynamics of CAF1-p180 or CAF1-p75 (Figures 3A, 3B, and S2). Confirming the specificity of our antibodies, following

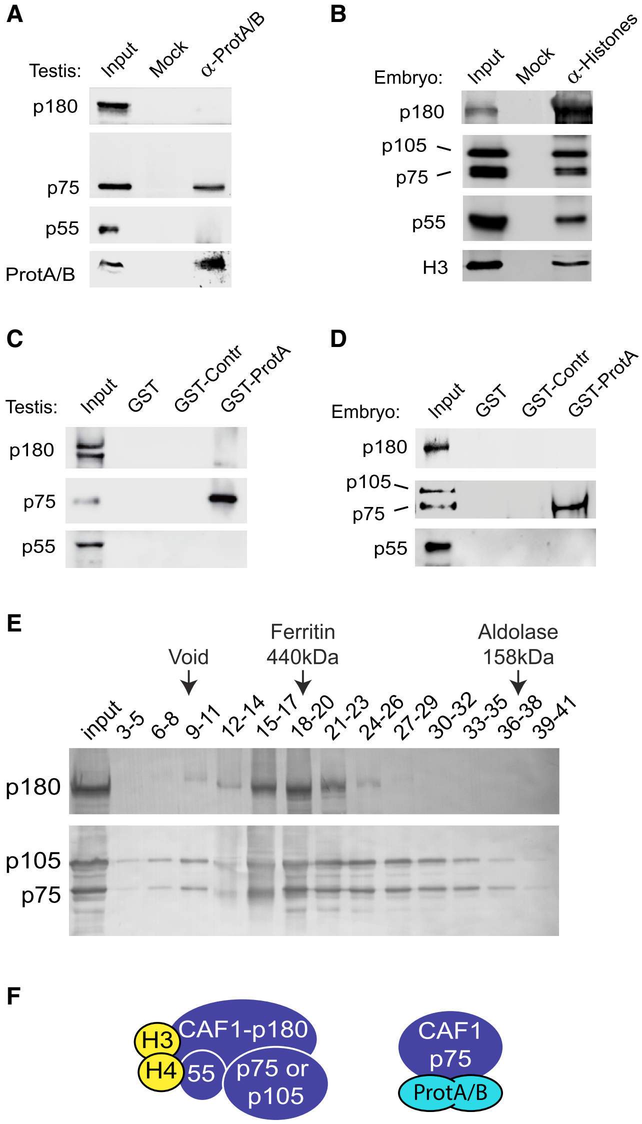

Figure 2. CAF1-p75 Binds Protamines

(A) CAF1-p75, but not the other CAF1 subunits, coimmunoprecipitates with the protamines. Coimmunoprecipitations (coIPs) from testis extract with antibodies directed against protamines. Bound proteins were resolved by SDS-PAGE and detected by immunoblotting. A total of 10% of input is loaded.

(B) All four CAF1 subunits coimmunoprecipitated with histones. CoIPs from embryo extract with antibodies against the core histones are shown.

(C) Immobilized GST-tagged protamine A (GST-ProtA), GST, or GST fused to a portion of an irrelevant protein (CG2982; GST-Contr) was incubated with testis extract. Following extensive washes with a buffer containing 200 mM KCl and 0.1% NP40, bound material was resolved by SDS-PAGE and analyzed by protein immunoblotting. Only CAF1-p75 binds ProtA.

(D) Shown is a similar GST pull-down experiment using an embryo extract.

(E) Subpopulations of endogenous CAF1-p75 and CAF1-p105 are not associated with CAF1-p180 in embryo extract analyzed by Sephacryl S-300 gel filtration chromatography. Eluted fractions were resolved by SDS-PAGE and analyzed by immunoblotting. Input (embryo extract), eluted fractions, voided volume, and elution of markers are indicated.

(F) Summary: the full CAF1 complex binds histones, but only CAF1-p75 binds protamines.

knockdown of either CAF1-p75 or CAF1-p180, the targeted protein was no longer detectable. These results suggest that CAF1-p180 is required for the genomic association of CAF1-p75 during spermatogenesis.

CAF1-p75 Is a Protamine-Loading Factor

The physical interaction between CAF1-p75 and protamines and their concomitant assembly into sperm chromatin suggested a functional relationship between these proteins. In particular, CAF1-p75 might act as a molecular chaperone that mediates protamine-based chromatin assembly during spermatogenesis. To test this idea, we examined the effect of CAF1-p75 depletion on protamine deposition (Figure 3C). Our antibodies revealed the protaminization of the paternal genome at the late canoe through mature sperm stages in wild-type and CAF1-p55 depleted testes. In the absence of CAF1-p75, however, protamine association with chromatin could no longer be detected. Likewise, depletion of CAF1-p180 caused failed protamine incorporation. Thus, CAF1-p75 and CAF1-p180 are required for the histone-to-protamine switch. The role of CAF1-p180 is most likely indirect as it does not bind protamines and dissociates from chromatin prior to or during protamine deposition (Figure 1). CAF1-p180 is, however, required for CAF1-p75 association with DNA (Figure 3A), which is a plausible explanation for its requirement for protamine incorporation into chromatin.

One possible explanation for the role of CAF1-p180 and CAF1-p75 in the histone-to-protamine switch could be a role in histone eviction. However, depletion of CAF1-p75, CAF1-p180, or CAF1-p55 had no effect on histone removal (Figure 3D). Thus, CAF1 is not required for histone eviction during spermatogenesis. We also raised antibodies directed against Mst77F, another key component of mature sperm chromatin that is deposited during the histone-to-protamine transition. Immunostainings showed that the stable incorporation of Mst77F into sperm chromatin was not affected by the loss of any CAF1 subunit (Figure 3E). Thus, CAF1 subunits are not involved in histone removal or Mst77F incorporation. Finally, immunoblotting showed that loss of either CAF1-p180 or CAF1-p75 did not have an appreciable effect on protamine levels (Figure 3F). Taken together, these results suggest that CAF1-p180 and CAF1-p75 function in a temporal hierarchy that mediates the protamine-based packaging of the paternal genome. Because it binds protamines and is required for their stable deposition onto DNA, we consider CAF1-p75 a protamine-loading factor.

CAF1-p75 and Protamine Deposition onto the Paternal Genome Is Mutually Dependent

CAF1-p75 does not simply hand off protamines onto the paternal DNA but is itself a component of mature sperm (Figure 1). Protamines and CAF1-p75 bind each other and are incorporated concomitantly at the late canoe stage. To test if the deposition of CAF1-p75 might be dependent on protamines, we analyzed this process in *Drosophila* lacking both ProtA and ProtB (*prot*Δ; Rathke et al., 2010). Immunostaining of developing sperm chromatin revealed the absence of CAF1-p75 association with sperm DNA in *prot*Δ animals (Figures 4A and S3). In contrast, CAF1-p180 binding to chromatin, which occurs prior to protamine incorporation, remained unaffected (Figure 4B). Likewise,

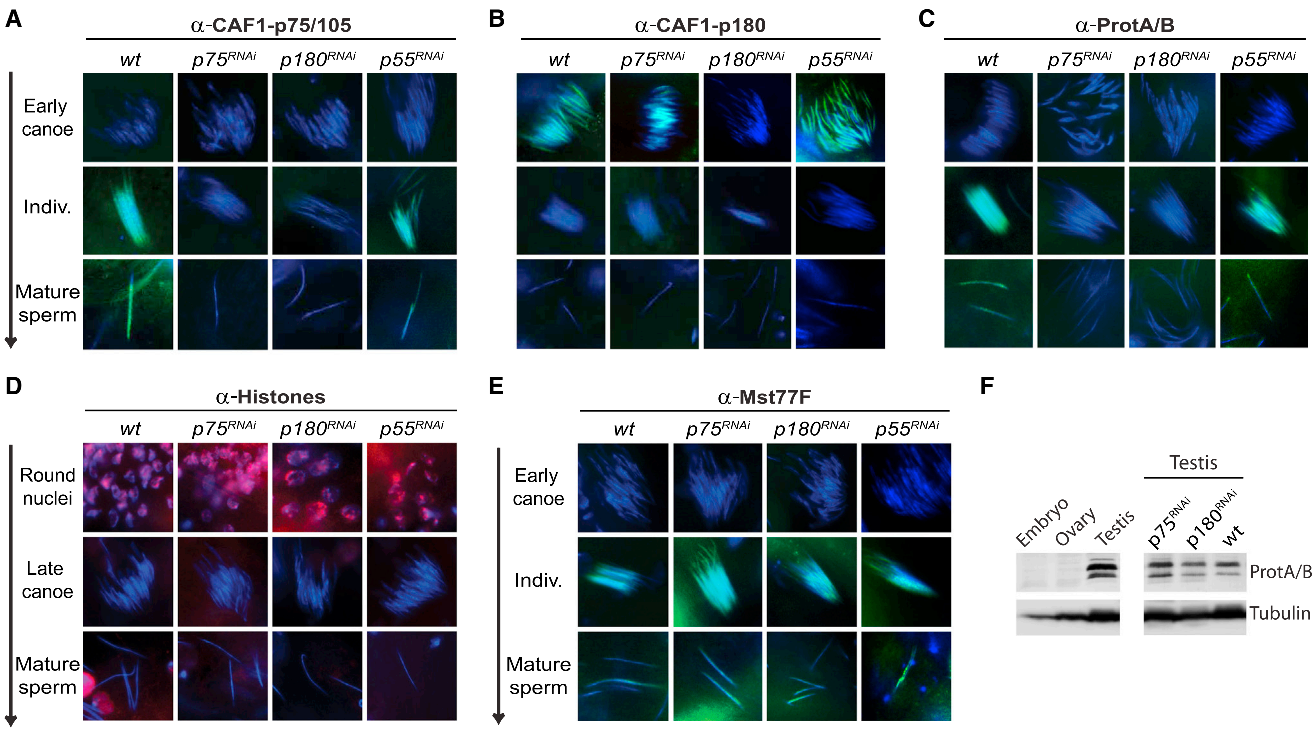

Figure 3. CAF1-p75 Is a Protamine-Loading Factor

(A) CAF1-p75 association with sperm chromatin is dependent on CAF1-p180. Binding to the paternal genome was monitored by immunofluorescence using α-CAF1-p75 antibodies in either wild-type testis (wt) or following C135-driven, RNAi-mediated depletion of CAF1-p75 (C135 > $p75^{RNAi}$), CAF1-p180 (C135 > $p180^{RNAi}$), or CAF1-p55 (C135 > $p55^{RNAi}$).

(B) CAF1-p180 association with sperm chromatin is independent of CAF1-p75. Analysis as described above using α-CAF1-p180 antibodies.

(C) CAF1-p75 and CAF1-p180 are required for protamine deposition. Endogenous protamines were monitored using α-ProtA/B antibodies.

(D) Histone removal is not affected by depletion of CAF1-p75, CAF1-p180, or CAF1-p55.

(E) Mst77F assembly onto sperm chromatin is independent of CAF1-p75, CAF1-p180, or CAF1-p55. Images of separate channels are shown in Figure S2.

(F) Western immunoblotting analysis of ProtA/B in extracts from embryos, dissected ovaries, wild-type testes, or testes in which CAF1-p75 or CAF1-p180 has been depleted by RNAi.

See also Figure S2.

CAF1-p180 removal from the DNA is not affected by the absence of protamines. Confirming earlier observations for ectopic Mst77-eGFP (Rathke et al., 2010), we found that endogenous Mst77F association with the paternal genome occurred normally in *protΔ* animals. We conclude that CAF1-p75 and protamine deposition onto the paternal genome is mutually dependent. These results reinforce the physical and functional association between CAF1-p75 and protamines.

DISCUSSION

The packaging of the male genome in sperm cells is fundamentally different from the nucleosome-based organization in somatic cells (Braun, 2001; Jayaramaiah Raja and Renkawitz-Pohl, 2005; Kimmins and Sassone-Corsi, 2005; Sassone-Corsi, 2002). Protamines and other sperm nuclear basic proteins that replace histones in sperm cells organize the genome into a toroidal structure that has no resemblance to a nucleosomal array. Compared to typical somatic cells, the sperm nuclear volume is condensed by more than two orders of magnitude and the protamine-based chromatin structure is not conducive to transcription or DNA replication. In spite of these differences, we found that the p180 and p75 subunits of the classic replication-coupled nucleosome assembly factor CAF1 are also required for protamine deposition during spermatogenesis. Thus, the same molecular machinery is utilized to create two inherently different forms of chromatin.

CAF1 functions differently when it mediates either histone or protamine deposition onto DNA. During DNA-replication-dependent nucleosome assembly, the CAF1 complex acts as a unit. During protamine deposition, CAF1-p180 and CAF1-p75 function in a temporal hierarchy whereas CAF1-p55 does not play a role. The transition from nucleosome-based to protamine-based chromatin occurs after meiosis and mitotic stages, when there is no DNA replication. Thus, the role of CAF1 in protamine deposition is replication independent. Importantly, histone removal during spermatogenesis is independent of CAF1 or protamine deposition. CAF1-p180 binds nucleosome-based paternal chromatin from the round nuclei through early canoe stages. Between early and late canoe stages, when the histone-to-protamine transition takes place, CAF1-p180 dissociates and CAF1-p75 binds chromatin and stays incorporated.

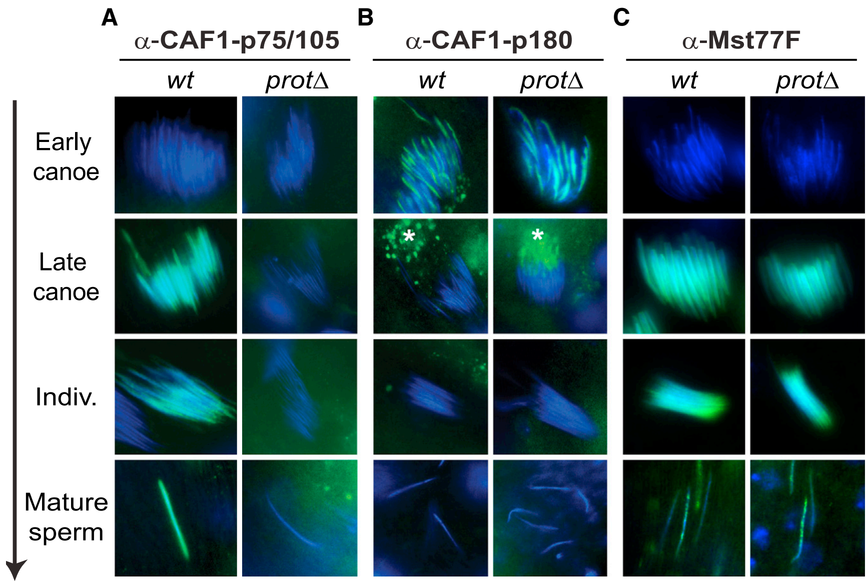

Figure 4. CAF1-p75 and Protamine Binding to the Paternal Genome Is Interdependent

(A) CAF1-p75 binding to the paternal genome was monitored by immunofluorescence in either wild-type testis (*wt*) or testis from flies lacking ProtA and ProtB (*prot⊿*).

(B) CAF1-p180 binding to chromatin is unaffected in *prot⊿* flies. "*" indicates the staining of an unknown structure separated from the DNA.

(C) Normal association of MST77F with the paternal genome is normal in *prot⊿* flies.

For all panels, images of separate channels are shown in Figure S3.

CAF1-p180 is, however, crucial for CAF1-p75 incorporation. CAF1-p75 binds the protamines and is required for their deposition. In turn, CAF1-p75 incorporation into sperm chromatin requires the protamines, emphasizing the intertwined roles of these factors. Thus, CAF1-p75 can be considered a protamine-loading factor or chaperone.

In contrast to mammalian protamines, in *Drosophila* ProtA and ProtB are dispensable for individualization and male fertility, although they help to protect the paternal genome from mutagens (Rathke et al., 2010). Likewise, loss of CAF1-p75 does not affect male fertility (data not shown). Mst77F, another spermatid-specific small basic protein, which shows homology to linker histones and mammalian HILS1, is also a component of mature sperm chromatin. Importantly, Mst77F is required for sperm chromatin compaction, nuclear shaping, and male fertility (Rathke et al., 2010). Therefore, the identifications of potential Mst77F chaperones will be important for our understanding of reprogramming of the paternal genome during spermatogenesis.

In summary, we uncovered a functional interaction between the histone chaperone CAF1-p75 and protamines during spermatid maturation. We conclude that although protamine-based chromatin is structurally unrelated to nucleosomal chromatin, their assembly involves components of the same cellular machinery. Our finding that CAF1-p75 is a protamine-loading factor emphasizes that histones are not the only substrate of histone chaperones.

EXPERIMENTAL PROCEDURES

Drosophila Stocks and Crosses

The C135-GAL4 enhancer-trap line (B6978) and RNA interference (RNAi) lines for CAF1-p180 (B28918) and CAF1-p55 (B31714) were obtained from the Bloomington Stock Center; the RNAi line for CAF1-p105/75 (v20270) was obtained from the Vienna RNAi Center. The protamine null line has been described previously (Rathke et al., 2010). All crosses were carried out at 25°C, and the embryos carrying the RNAi and driver were developed at 28°C.

Antibodies and Protein Purification

Polyclonal antibodies were generated by immunizing guinea pigs with purified glutathione S-transferase (GST) fusion proteins expressed in *E. coli* (Chalkley and Verrijzer, 2004). The following antigens were used: Mst35Ba (amino acids 6–123), Mst35Bb (6–127), CAF1-p180 (800–1,150), Mst77F (1–215), CAF105/75 (431–583), CAF1-p105-specific (620–740), and purified core histones (Katsani et al., 2001). Rabbit polyclonal antibodies against CAF1-p105 and CAF1-p55 have been described previously (Moshkin et al., 2009). Extract preparations, coimmunoprecipitations, and GST pull-downs were performed as described elsewhere (Chalkley et al., 2008; Chalkley and Verrijzer, 2004). Size-exclusion chromatography was performed as described previously (Moshkin et al., 2009). Embryo nuclear extracts were prepared from 0- to 12-hr-old *Drosophila* embryos. For coimmunoprecipitations, beads were washed three times with HEMG buffer (25 mM HEPES-KOH [pH 7.6], 0.1 mM EDTA, 12.5 mM $MgCl_2$, 10% glycerol, 0.1% NP-40, and a cocktail of protease inhibitors) containing 200 mM KCl (HEMG/200), three times with HEMG/400, and two times with HEMG/200 lacking NP-40. Protein isolation for mass spectrometric analysis included three washes with HEMG/600 and was performed as described elsewhere (Moshkin et al., 2009).

Immunofluorescence

Testes were dissected from 4-day-old males and collected in PBS-Triton 0.1%, fixed with 4% formaldehyde for 10 minutes at room temperature, rinsed three times for 10 minutes in TBST (50 mM Tris HCl [pH 8], 150 mM NaCl, 0.1% Triton), and incubated with primary antibodies overnight at 4°C. Next, testes were washed in TBST and stained with secondary Alexa Fluor antibodies (Invitrogen). Following three washes with TBST, testes were mounted in Vectashield containing DAPI. Primary antibodies were used at 1:500 dilution and secondary antibodies at 1:200.

SUPPLEMENTAL INFORMATION

Supplemental Information includes three figures and one table and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2013.06.002.

ACKNOWLEDGMENTS

This work was supported in part by the Netherlands Proteomics Centre (to C.P.V.) and the International Graduate School Marburg-Rotterdam TRR81.

Received: January 28, 2013
Revised: April 25, 2013
Accepted: June 1, 2013
Published: June 27, 2013

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